Morphological and physiological adaptations to waterlogging by *Eucalyptus* seedlings from the semi-arid Pilbara, Western Australia

S K Florentine* & J E D Fox

School of Environmental Biology, Curtin University of Technology, GPO Box U 1987, Perth WA 6845
*current address: School of Science, University of Ballarat, PO Box 663, Ballarat VIC 3350
email: s.florentine@ballarat.edu.au

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Abstract

This study was undertaken to investigate the adaptation to long term waterlogging of semi-arid eucalyptus species. Long-term waterlogging of *Eucalyptus victrix* seedlings significantly increases seedling stem diameter. Flooding reduces photosynthesis, transpiration and stomatal conductance. Flooding does not increase shoot fresh or dry weight of 4-, 8- or 17- week old seedlings. Leaf emergence may be stimulated for flooded seedlings compared with unflooded seedlings. Root dry weight is not significantly greater for 17- week old flooded plants than 13- week old seedlings. We suggest that maintenance of a high root/shoot ratio is a drought adaptation. Furthermore, a comparative study of flood tolerance in semi-arid eucalypt species suggests that those species intolerant of flooding seldom express morphological adaptations and fail to recover from physiological damage. Flooding significantly reduced the transpiration rate and stomatal conduct of all three species. Diurnal transpiration, stomatal conductance and leaf water potential of *E. terminalis* and *E. leucophloia* were significantly different between treatment (flooding) and control seedlings.

Keywords: *Eucalyptus*, flooding, floodplain, semi-arid, *E. victrix*, *E. terminalis*, *E. leucophloia*

Introduction

The large Australian genus *Eucalyptus* has species adapted to a wide range of climatic and edaphic conditions (Goor & Barney 1968). Vernacular names often indicate tolerance of environments subject to flooding; *e.g.* flooded gum *E. grandis*, swamp gums *E. camphora* and *E. ovata*, and swamp mahogany *E. robusta*. These are all species that can withstand some degree of inundation (Ladiges & Kelso 1977; Clemens & Pearson 1977; Kozlowski *et al.* 1991). Some species (*e.g. E. camaldulensis*) are dependant on periodic flooding with seed germinating on flooded areas following flood recession (Parsons *et al.* 1991). We examine and compare here the flood tolerance of three *Eucalyptus* species found in the semi-arid Pilbara region, one of which grows in flood prone areas and two which grow in non-flood prone areas.

Within the large genus *Eucalyptus*, species of the subgenus *Monocalyptus* are less water and frost tolerant than are *Symphyomyrtus* species (Noble 1989; Davidson & Reid 1987). No *Monocalyptus* species have been shown to tolerate waterlogged conditions (McComb *et al.* 1989; van der Moezel & Bell 1990; Bell *et al.* 1994), although of two south-west Western Australian *Monocalyptus*, *E. marginata* (jarrah) is very intolerant to waterlogging (Davison & Tay 1985) whereas *E. patens* is often found in low-lying areas (Bell & Williams 1997). Species intolerant of waterlogging generally show no morphological changes and no recovery of gas exchange during exposure to the waterlogged condition (Tang & Kozlowski 1982). Species tolerant of waterlogging often show reduced stomatal conductance and transpiration but tend to recover immediately after adventitious roots are formed (Gomes & Kozlowski 1980a,b).

Symphyomyrtus species that occur on or adjacent to the Fortescue floodplain, in the semi-arid Pilbara region include *E. victrix*, *E. camaldulensis* var *obtusa* Blakely and *E. leucophloia* Brooker. Trees that occur in flood plain environments experience exposure to flooding that may be regular or irregular and of varied duration and frequency. *E. victrix* L Johnson & K Hill forms open, grassy woodlands in the flood plain of the Fortescue River valley (Xin *et al.* 1996). This area is subject to summer flooding from cyclones or heavy thunder storm activity between January and March. Depending on the topography of the woodland, flood water may remain for a month or more.

The present study was undertaken to examine aspects of tolerance to flooding by *E. victrix* seedlings using plants of different ages from germination. This paper compares three Pilbara eucalypts (*E. victrix*, *E. terminalis* and *E. leucophloia*) that differ in ecological habitat. *E. victrix* is confined to typical floodplain sites, *E. terminalis* occurs on river banks but is also scattered on hillsides, whereas *E. leucophloia* occurs mostly on stony hills. Based on their ecological distribution, it is hypothesised that seedlings of the non-floodplain *Eucalyptus* species *E. leucophloia* and *E. terminalis* are less tolerant in terms of ecophysiology and seedling growth to flooding than the typical flood plain species *E. victrix*. We test the hypothesis that *E. victrix* seedlings will not show growth-related changes in CO_2 assimilation rates when exposed to prolonged flooding.

Methods

Effects of flooding

Seedlings of *E. victrix* were germinated and grown under similar conditions until imposition of flooding treatment on some seedlings at 4, 8, 13 and 17 weeks after planting. Seed was extracted from mature fruit capsules of *E. victrix* collected in February 1995, from the coolibah woodland of the Fortescue near Ethel Creek (122° 54'S, 120° 10'E), and were stored in a sealed jar at laboratory temperature (about 21° C). Aliquots of seed were sown onto sterilised coarse sand on Aug–Sept 1995. Uniform size (5-6 cm height) seedlings were transplanted into cylindrical pots (150 mm height and 80 mm wide), containing clay soil collected from the coolibah woodland. All the pots were kept in a glass house and watered 3 to 4 times a week. Two weeks after, to simulate the natural condition, seedlings were moved to an open area.

Cylindrical pots of 13 cm diameter were filled with coolibah woodland soil (red clay loam, pH 7) and the bottom openings sealed with plastic draining tapes. Seedlings were transplanted into pots at the 2-4 leaf stage, approximately three weeks after sowing. Plants were maintained in a shade house until Dec 1995 when they were placed in full sunlight. After five days acclimatisation, flooding treatment commenced. The plants were then 13- or 17-weeks old at the start of flooding, with mean heights of 14.1 and 19.0 cm respectively. Fibreglass tanks of 2.5x0.5x0.5 m were filled with rainwater to a depth of 1 cm above the pot soil level (12 plants of 13-week old; 8 plants of 17-week old). Rainwater was added daily to maintain the level. Control plants were placed adjacent to the tanks and these were maintained in a freely drained condition and were watered to excess three times a week. All plants were in full sun. Further seed was sown and seedlings prepared as before, to give 4- and 8-week old seedlings. Measurements were made on seedling height, leaf number, and leaf dimensions (length and width). New leaf emergence and leaf death were also noted. Any changes in seedling morphology, particularly stem swelling, leaf colour changes and development of adventitious roots were recorded at weekly interval. All plants were harvested 32 weeks after flooding of the 13- and 17- week sets and carefully removed from pots. Fresh weights of root and shoot were obtained. Plants were then put in separately labelled paper bags and dry weights obtained after 24 hr at 105 °C.

Leaf gas exchange

Leaf gas exchange measurements (net photosynthesis, stomatal conductance, transpiration) were made on the 13- and 17-week old seedlings on fully expanded individual leaves from each plant. Measurements commenced 8 days after the start of flooding and continued for 6 weeks using a portable gas exchange system (model LCA-3, Analytical Development, UK) with leaf chamber attachment (Parkinson PLC-031 3B). Data were collected under ambient conditions with photosynthetic active radiation (PAR; 400–700 nm) >500 μmol $m^{-2} s^{-1}$, consistently between 1200 and 1300 hrs.

Comparison of flooding tolerance

Seeds of *E. victrix*, *E. terminalis* (desert blood wood) and *E. leucophloia* (migum/snappygum) were collected from sites near Newman (23° 21' S, 119° 44' E), Western Australia on 27 March 1997. Air-dried, but uncleaned seeds were stored in air-tight bottles and kept at room temperature until use. On 25 July 1997, seeds were sown in sterilised coarse sand in seedling trays at the Field Trial Area of Curtin University. On 15 August, 50 uniform sized (5-6 cm high) seedlings from each species, were transplanted into cylindrical pots containing clay soil collected from a coolibah woodland. All pots were kept in a glass house and water was added 3 to 4 times a week. On 21 January 1998 (to simulate the natural condition) uniform size seedlings (29 seedlings of *E. victrix* and *E. leucophloia* and 35 seedlings of *E. terminalis*) were selected. Roots of all three of the species had penetrated through the basal holes. For uniformity all plants were removed carefully from pots and roots were trimmed. The seedlings were then placed in the middle of plastic pots (13 cm dia) and any gaps were filled with coolibah woodland soil. Pots were watered and left in the glass house for one week. Plants were then taken outside and randomly divided into two groups (control and treatment). The flooding treatment involved by placing seedlings randomly inside three fibre-glass tanks (2x2 m) filled with rain water. The water level was maintained at 15 to 20 mm above the soil surface. Control seedlings were kept on a table beside the tanks. Daily maxi and min ambient temperatures were recorded throughout the experiment.

Gas exchange measurements were made at irregular intervals, commencing from two weeks after the start of waterlogging. In addition, diurnal ecophysiological and environmental measurements were made on 10 April 1998. Gas exchange measurements (net photosynthesis, transpiration, stomatal conductance and internal CO_2 concentration) were recorded for five seedlings of each of the three *Eucalyptus* species from both control and waterlogged treatment. Recordings were made every 3 hours between 0600 hours and 1800 hours local time using an open portable gas exchange system. Diurnal leaf water potentials (Ψ) of excised leaves were determined using a pressure chamber (Model Mk 3005, Soil Moisture Equipment, CA; Scholander *et al.* 1965) at 3 hourly intervals, on the same seedlings as gas exchange measurements were taken. All the leaves used for the water potential study were kept in labelled bags and added to respective seedlings at the end of the experiment for dry weight measurements.

All plants were harvested on 11 April 1998, 65 days after the waterlogging treatment commenced. Each plant was carefully removed from its pot, washed, surface dried between paper towels, and placed in separately labelled bags. At this time observations were made of any morphological adaptations to waterlogging: roots (mainly adventitious root), shoots (hypertrophy), leaf (colour changes; Royal Horticulture Society Colour Chart) and soil surrounding root parts. Samples were stored at 6 °C and the following day projected leaf area, length and width were determined using a digital image analyser (DIAS, Delta-T Devices). Plants were then oven-dried at 105 °C for 24 hr and dry weights were measured separately for shoots and roots.

Statistical analyses

Data were analysed by one-way ANOVA using SuperANOVA software program (Abacus Concepts, CA). Residual plots of each ANOVA were obtained to examine homogeneity of variance. Based on residual plots, data were transformed to log or square root as appropriate and reanalysed. The data presented here are of uniform means.

Results

Flooding of *E. victrix* seedlings

The net photosynthetic rate of 13-week control plants fluctuates between 7 and 15 μmol m^{-2} s^{-1} over the 50 day experimental period, whereas net photosynthetic rate of flooded plants declining to less than 5 μmol m^{-2} s^{-1} after two weeks flooding and remains below 7 μmol m^{-2} s^{-1} thereafter (Fig 1). The net photosynthetic rate of flooded and controlled 13-week old seedlings was significantly different from the first measurements. After waterlogging, the 17-week old plants showed no significant difference in net photosynthetic rate but the effect was highly significant during the subsequent measurements.

Changes in stomatal conductance were generally similar to those for photosynthesis, with a highly significant difference for 13-week old flooded plants but no significance difference for 17-week old ones (Fig 1). Control plant gas exchange values also gradually decreased during the study period, apart from a fluctuation at 40 days for the 13-week old seedlings. Fifteen days after the flooding treatment began, most values for flooded plants were below those of the corresponding control, and remained less.

Transpiration rates of both 13- and 17- week sets were similar for control and flooded plants up to 21 days (Fig 2). However, this changed in both 13 and 17-week old seedlings. There was a clear significant difference after 13-weeks, with higher rates for controls.

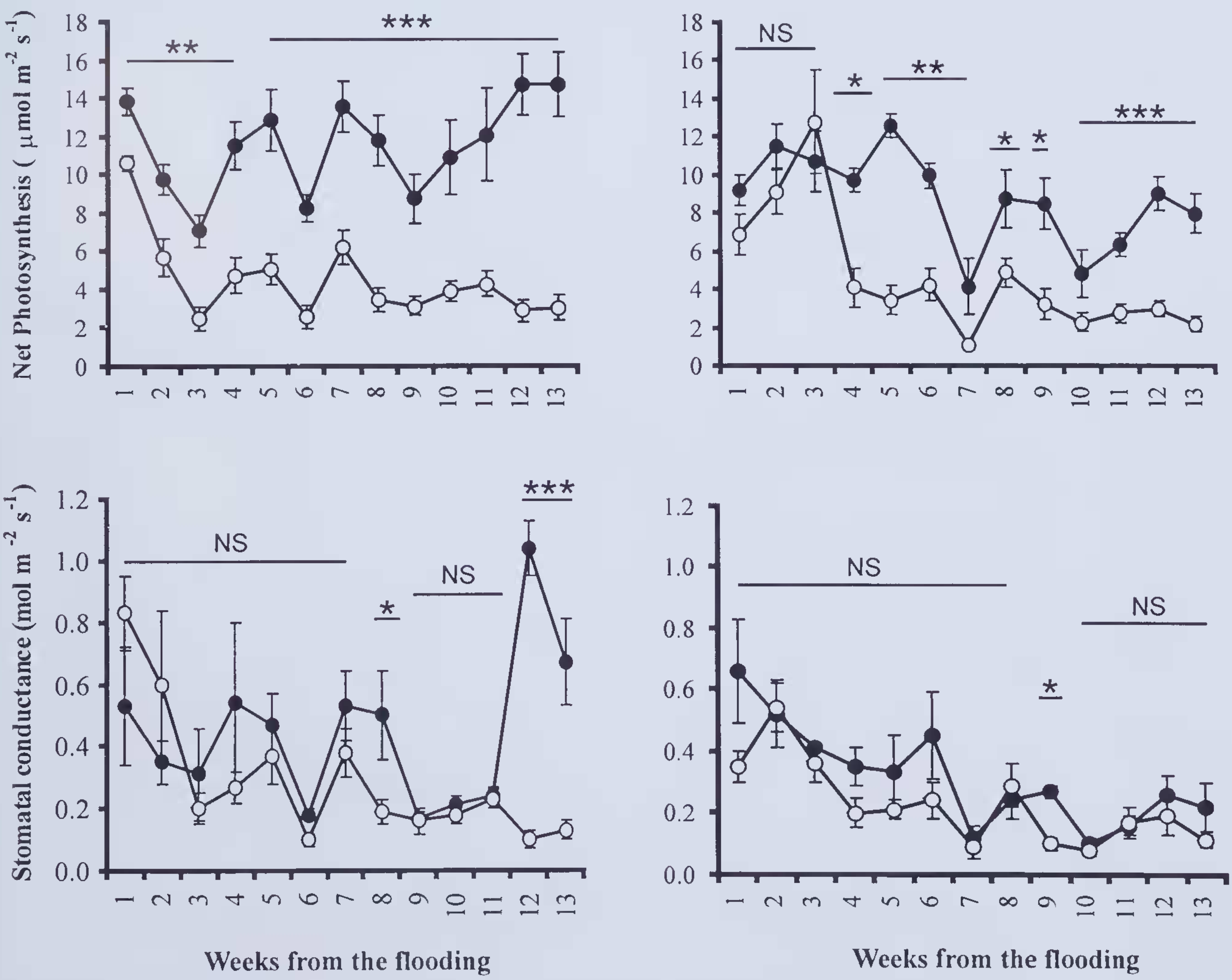

Figure. 1. Net photosynthesis (top), and stomatal conductance (bottom) rate for 6 weeks following flooding on 13 (left) and 17 (right) week-old seedlings of *E. victrix*; controls (•) and flooded (o). NS indicates no significant difference; * indicate samples are significantly different at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

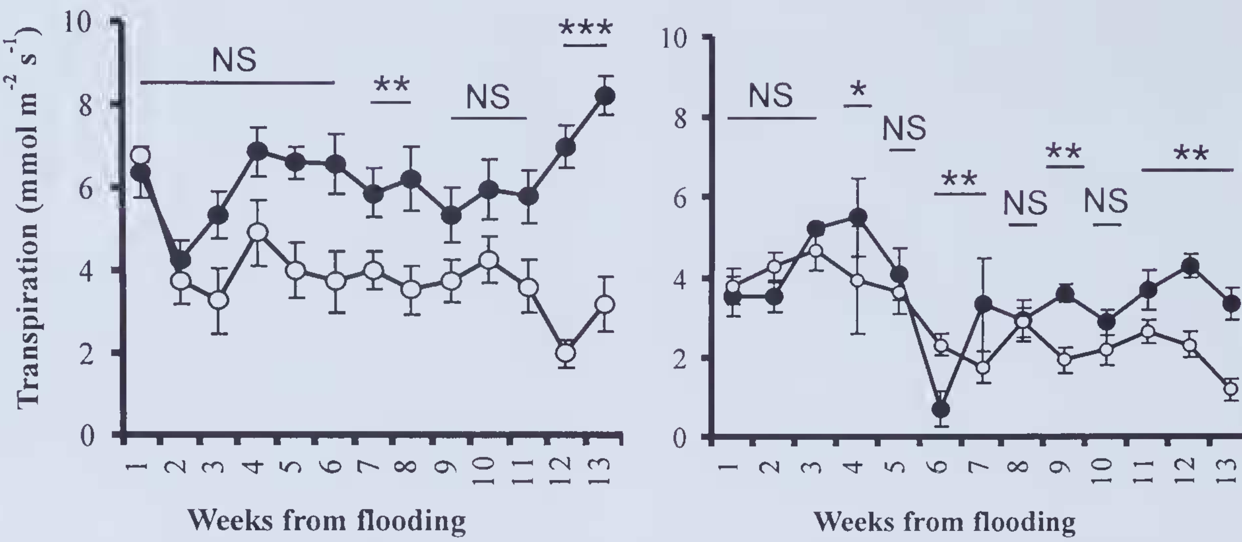

Figure 2. Transpiration rate, for 6 weeks following flooding on 13 (left) and 17 (right) week-old seedlings of *E. victrix*; controls (•) and flooded (o). NS indicates no significant difference; * indicates significant differences at $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Leaf colour changed from 16 days after waterlogging. Leaves initially became reddish-brown; some became yellowish after 32 days. After 47 days, submerged stems had begun to show splitting in the 17-week set. Adventitious root development was observed from 42 days in the 13-week old seedlings and from 62 days in the 17-week old plants. Mean total leaf areas (mm^2) of the 17-week plants at final harvest were; control 789 ± 424 (SD) and flooded 1063 ± 427 (SD). Flooded seedlings shed an average of two leaves each. New leaf formation appeared to be continuous in both flooded and control plants but was more prolific in flooded plants.

In all four-seedling age sets, the mean shoot height at harvest did not differ significantly between control and flooded seedlings (Tables 1 and 2). There was no significant difference in shoot dry weights even though

Table 1

Harvest details of 4- and 8- week old *Eucalyptus victrix* seedlings after flooding. $*P < 0.05$; $**P < 0.005$; $*** P < 0.001$; NS = not significant. Data are mean ± SE.

Treatment	Age	n	Shoot height (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Stem diameter (mm)	Number of adventitious roots
Flooded	4	11	12.91 ± 0.95	0.80 ± 0.08	26.40 ± 1.80	0.41 ± 0.70	3.16 ± 0.37	14.72 ± 1.01
Unflooded	4	4	10.88 ± 0.58	0.59 ± 0.05	57.40 ± 5.70	1.01 ± 0.07	2.20 ± 0.13	0.00
Flooded	8	12	21.90 ± 1.34	2.42 ± 0.29	23.26 ± 6.22	0.94 ± 0.19	5.68 ± 0.33	13.41 ± 1.16
Unflooded	8	4	21.77 ± 5.11	1.83 ± 0.45	40.50 ± 7.00	2.54 ± 1.40	3.70 ± 0.56	0.00
P values								
Flooding			NS	NS	***	***	***	***
Age			***	***	**	***	***	NS
Flooding x Age			NS	NS	*	NS	NS	NS

Table 2

Harvest details of 13- and 17-week old *Eucalyptus victrix* seedlings. $*P < 0.05$; $** P < 0.01$; $*** P < 0.001$; NS = not significant. Data are mean ± SE.

Treatment	Age	n	Shoot height (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Stem diameter (mm)	Number of adventitious roots
Flooding	13	12	16.66 ± 0.99	1.54 ± 0.21	20.56 ± 2.28	3.02 ± 0.75	4.70 ± 0.23	10.00 ± 1.04
Unflooded	13	4	16.20 ± 0.75	0.84 ± 0.17	38.75 ± 1.63	2.33 ± 0.75	3.05 ± 0.64	0.00
Flooded	17	7	22.09 ± 2.22	4.71 ± 0.39	27.19 ± 1.02	8.17 ± 1.50	7.40 ± 0.78	10.14 ± 1.40
Unflooded	17	4	21.05 ± 1.80	4.41 ± 0.31	27.78 ± 3.10	4.37 ± 0.68	4.31 ± 0.29	0.00
P values								
Flooding			NS	NS	***	NS	***	***
Age			**	***	NS	**	**	NS
Flooding x Age			*	NS	**	NS	NS	NS

13-week old flooded seedlings have almost twice the mean weight of the control (Table 1). Root length, root mass, stem diameter and number of adventitious roots were significantly different (P <0.0001) in 4 and 8- week old seedlings (Table 1). However, there was no significant difference in shoot height or dry mass. Harvest data of 13- and 17-week old seedlings indicated that flooding was associated with significant differences (P <0.0001) in root length, stem diameter and number of adventitious roots (Table 2). A significant interaction between flooding condition and seedling age was observed in shoot height and root length.

At harvest, all original root systems on waterlogged plants were soft and black, in stark contrast to the white-cream coloured adventitious roots. Flooding had the greatest effect on original seedling roots. The older sets the roots of flooded seedlings were black and decaying at the end of the experiment (32 weeks after). Just above the dead roots, prolific white adventitious roots were clearly observed in the 8 week seedlings.

Flooding tolerance

No plants died during the flooding experiment. Increase in height of specimens of all three *Eucalyptus* species subject to waterlogging was consistently slower than the controls (Table 3). Height growth of *E. victrix* was least affected by waterlogging, and no significant differences were observed for either *E. victrix* or *E. leucophloia*.

The number of intact leaves decreased within two weeks from initiation of waterlogging, particularly for *E. terminalis* (Table 3). At this stage, *E. leucophloia* and *E. victrix* showed symptoms of leaf epinasty in flooded seedlings. Leaf number increased for *E. victrix* for the first 23 days after flooding, followed by a gradual decline. Flooding effects were almost similar towards the end of the experiment for both *E. terminalis* and *E. leucophloia* where both species had reduced numbers of leaves attached to stems. At the end of the experiment (65 days after flooding) adventitious roots was found only for waterlogged *E. victrix* (3.60 ± 1.44, n = 9) with none formed in either *E. leucophloia* or *E. terminalis*.

Differences in shoot dry mass due to flooding were significant for both *E. leucophloia* (P = 0.05) and *E. terminalis* (P < 0.001; Table 4). The effect was most pronounced for *E. terminalis*; differences between waterlogged and control seedlings were also significant for *E. victrix* (P = 0.022). Analysis of whole plant dried mass indicated significant differences for all three species. The effect was more severe for *E. terminalis*. Analysis of shoot:root ratio showed differences for all three species.

Transpiration rates of *E. victrix* and *E. leucophloia* were similar between flooded and unflooded plants after 9 days (Fig 3). However, after 23 days transpiration was significantly lower for flooded plants. Transpiration was subsequently reduced in flooded *E. terminalis* and *E. leucophloia* but remained steady for *E. victrix*.

Stomatal conductance was significantly different between flooded and control plants after 9 days of flooding (Fig 3) with rates between 4.2 and 6.8 mol m^{-2} s^{-1} for unflooded plants. The range was only 0.3 to 1.1 m mol m^{-2} s^{-1} in all three species for flooded plants. Stomatal conductance remained relatively low for flooded *E. terminalis* and *E. leucophloia* but in contrast stomata started to reopen for *E. victrix* (stomatal conductance increased from 0.8 to 2.7 mol m^{-2} s^{-1}) by 40 days after flooding, and increased further by 60 days.

Diurnal patterns of temperature and PAR are given in

Table 3

Effect of waterlogging on height (cm), number of leaves and leaf area parameters for *E. victrix*, *E. terminalis* and *E. leucophloia*. Data are mean ± SD of tallest seedlings (n= 10) from each species for height, number of leaves and leaf area, leaf length, width and length:width ratio. NS indicates means are not significantly different; significant differences at P < 0.05*, P <0.01**, P< 0.001***.

	E. victrix		*E. terminalis*		*E. leucophloia*	
	Flooded	**Control**	**Flooded**	**Control**	**Flooded**	**Control**
Height (cm)						
09 days	21.2± 4.3	21.6 ± 3.3 NS	20.9 ± 1.2	22.9 ± 1.6**	19.6 ± 2.9	18.4 ± 3.7 NS
23 days	23.7 ± 4.8	24.2 ± 3.1 NS	21.9 ± 1.0	24.5 ± 1.9**	19.7 ± 2.8	18.9 ± 4.1 NS
31 days	24.1 ± 5.3	26.4 ± 3.0 NS	20.6 ± 2.1	24.5 ± 1.8**	20.0 ± 3.0	19.6 ± 4.4 NS
38 days	24.1 ± 5.7	27.2 ± 3.3 NS	21.0 ± 3.0	24.8 ± 1.8**	20.0 ± 2.8	20.0 ± 4.2 NS
59 days	25.2 ± 5.6	28.2 ± 2.9 NS	21.1 ± 3.1	25.6 ± 1.8***	20.1 ± 2.8	21.4 ± 4.6 NS
Number of leaves						
09 days	17.3 ± 2.1	17.3 ± 3.9 NS	18.0 ± 3.6	21.7± 3.8**	20.8 ± 3.0	18.2 ± 4.4 NS
23 days	17.9 ± 2.5	19.2 ± 5.3 NS	17.9 ± 4.1	21.3 ± 4.5NS	20.7 ± 2.7	21.8 ± 5.2 NS
31 days	17.2 ± 2.9	19.8 ± 5.3 NS	15.5 ± 5.1	21.4 ± 3.8**	18.2 ± 4.4	21.9 ± 5.0 NS
38 days	16.6 ± 1.6	21.3 ± 6.4*	15.0 ± 5.5	22.6 ± 3.9**	17.2 ± 3.1	23.3 ± 5.7**
59 days	15.5 ± 1.6	20.3 ± 5.7*	14.0 ± 4.9	21.9 ± 4.7**	13.2 ± 2.6	23.3 ± 5.1***
65 days	14.5 ± 3.0	19.1± 5.3*	12.9 ± 4.6	21.6 ± 6.4**	10.5 ± 2.6	23.6 ± 5.8***
Leaf area parameters						
Area (mm^2)	110. 5 ± 19.6	121.3 ± 10.3 *	84.0 ± 21.7	102.6 ± 10.5*	113.4 ± 22.5	138.1 ± 38.5 NS
Length (mm)	34. 8 ± 5.8	38. 6 ± 3.8*	34.0 ± 25.6	33.6 ± 5.7NS	24.7 ± 4.7	33.5 ± 4.0***
Width (mm)	16. 2 ± 2.2	17. 3 ± 1.7 NS	12.6 ± 3.4	17.2 ± 1.9**	20.1 ± 3.4	25.9 ± 3.6**
L:W ratio	2.1 ± 0.3	2.2 ± 0.15 NS	2.6 ± 1.5	1.9 ± 0.3 NS	1.2 ± 0.1	1.3 ± 0.0*

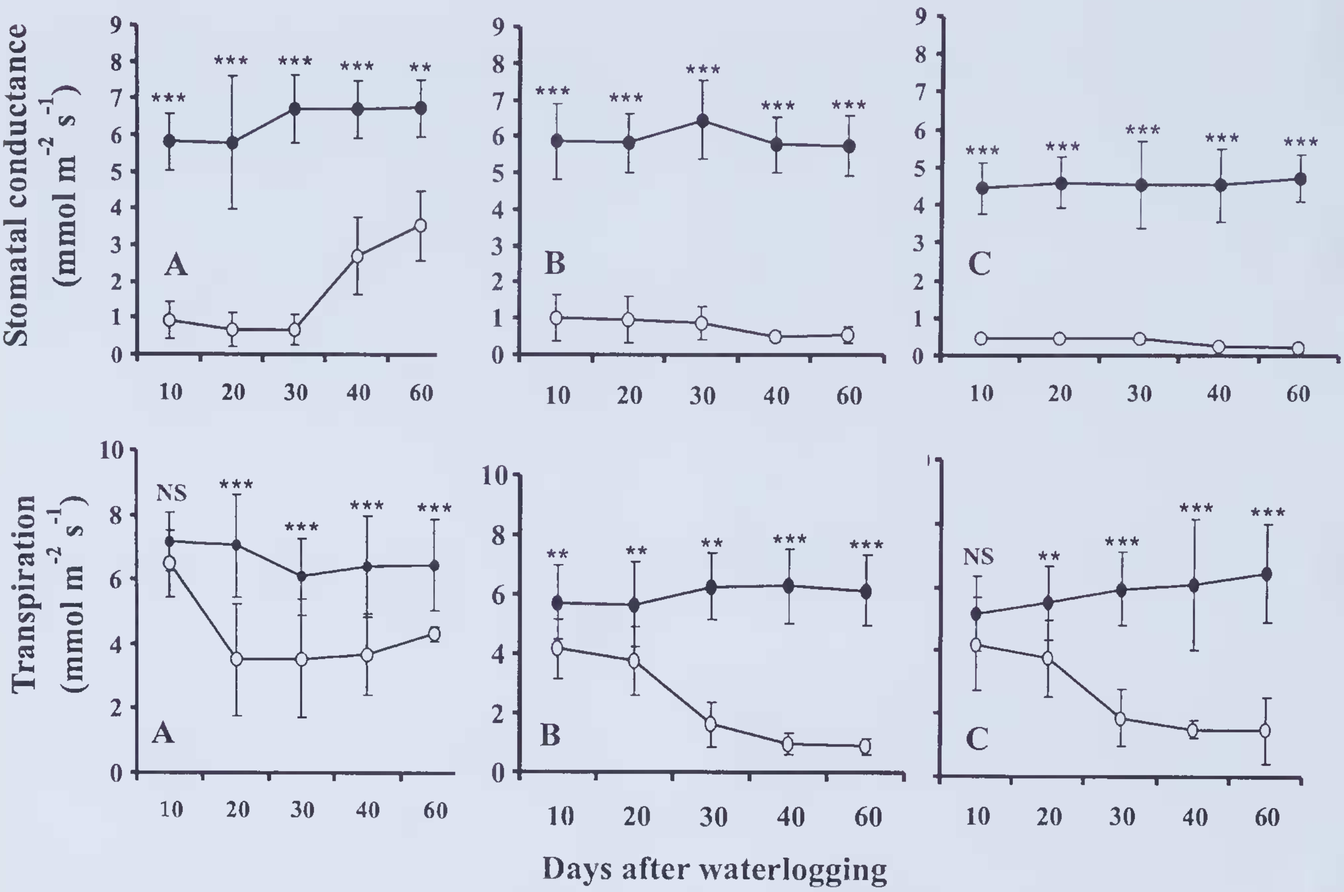

Figure 3. Changes in stomatal conductance and transpiration of *E. victrix* (A), *E. terminalis* (B) and *E. leucophloia* (C) of control (o) and flooded (•) and seedlings. The first measurements were taken 9 days after exposure to flooding. Vertical bars indicate standard error of means of five measurements. NS indicates no significant difference; * indicates samples are significantly different at $P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4

Mean (± SD) of shoot dry weight, root dry weight, whole plant and shoot/root ratio of tallest seedlings from each of *E. victrix*, *E. terminalis*, and *E. leucophloia* seedlings after 65 days of waterlogging. Significant difference are $P < 0.05^*$; $P < 0.01^{**}$; $P<0.001^{***}$. NS indicates means are not significantly different.

	E. victrix			*E. terminalis*			*E. leucophloia*		
Attribute	Flooded (n= 10)	Control (n= 10)	*P*	Flooded (n= 10)	Control (n= 10)	*P*	Flooded (n= 10)	Control (n= 10)	*P*
Shoot dry wt(g)	2.66 ± 0.64	3.33± 0.54	*	1.51 ± 0.63	3.05 ± 0.84	***	2.55 ± 1.03	4.18 ± 1.66	*
Root dry wt(g)	0.64 ± 0.22	3.54 ± 1.29	***	0.37 ± 0.13	2.92 ± 1.10	***	0.53 ± 0.24	2.22 ± 0.88	***
Whole plant dry wt(g)	3.30 ± 0.73	6.87 ± 1.33	***	1.88 ± 0.69	5.98 ± 1.33	***	3.08 ± 1.18	6.41 ± 2.46	**
Shoot: Root ratio	4.77 ± 2.31	1.05 ± 0.41	***	4.37 ± 1.84	1.23 ± 0.66	***	5.66 ± 3.05	1.94 ± 0.50	**

Fig 4. Diurnal transpiration of flooded *E. leucophloia* and *E. terminalis* plants was significantly lower ($P < 0.001$) than for unflooded seedlings. For *E. victrix* a marginally significant difference ($P = 0.043$) was observed at 1200 hr. The difference in transpiration rate of flooded and control *E. leucophloia* and *E. terminalis* varied from 5-7 mmol m^{-2} s^{-1} at each sampling time (Fig 4).

The diurnal stomatal conductance of control plants (Fig 4) for all three species exceeded 1.5 m mol m^{-2} s^{-1}, and that of water-logged seedlings was between 1.0 and 1.5 m mol m^{-2} s^{-1} during the early part of the day. For both waterlogged and control plants, stomatal conductance declined progressively soon after sunrise. However, rates started to recover from 1500 hr. Differences were not significant between waterlogged and control plants of all three species. Although diurnal mean leaf water potential (Ψ) of waterlogged seedlings was consistently more negative than that of control plants, differences were only

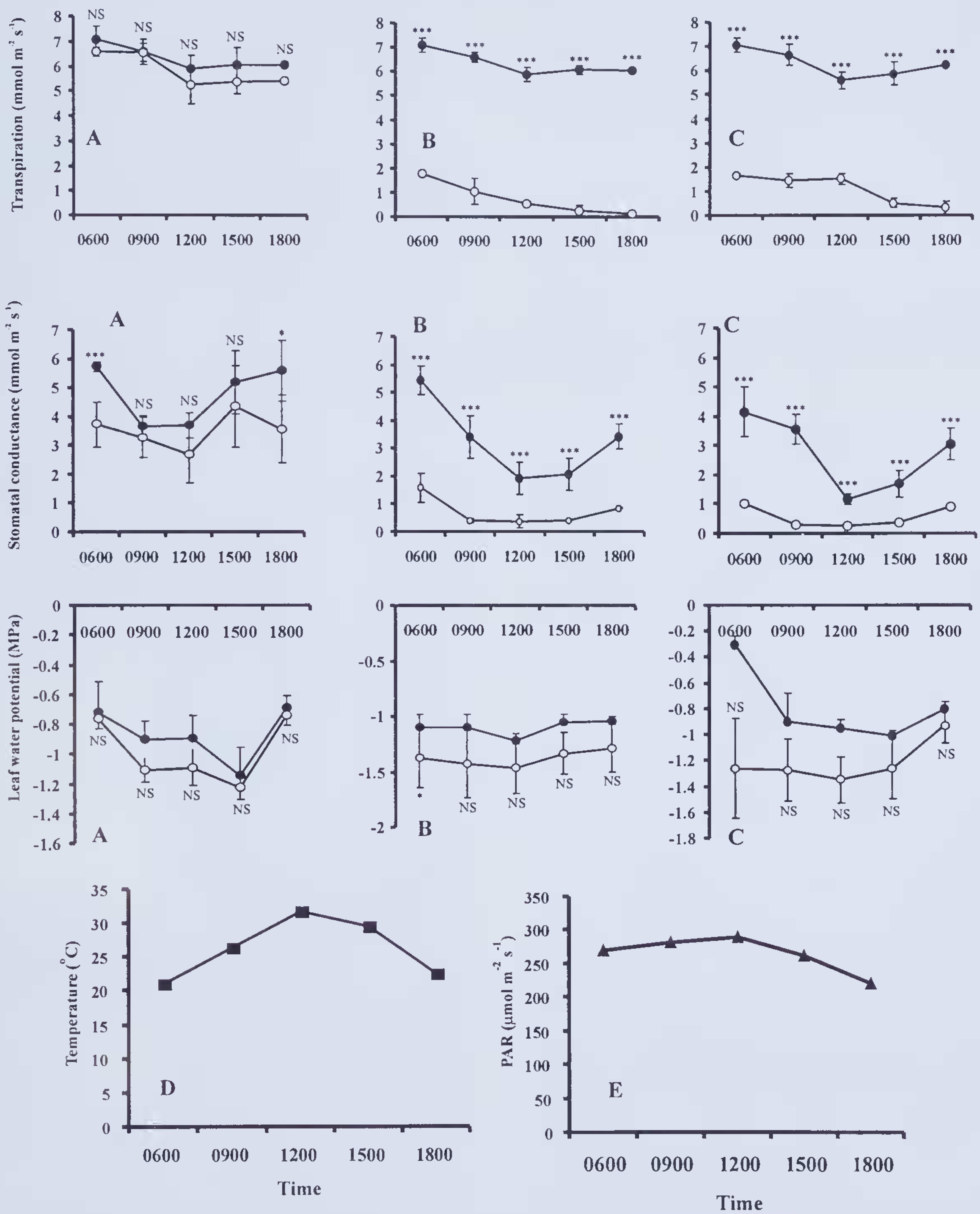

Figure 4. Diurnal patterns of transpiration, stomatal conductance and mean pre-dawn leaf water potential of control (•) and flooded (o) seedlings of *E. victrix* (A), *E. terminalis* (B) & *E. leucophloia* (C) after 65 days of flooding. Values are mean and standard error (n = 5). NS indicates no significant difference; * indicates samples are significantly different at $P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Temperature (D) and photosynthetic active radiation (E) are shown for the time of measurements.

significantly different for *E. leucopholia* at 0600 hr (P = 0.026) and *E. terminalis* at 1200 hrs (P = 0.002; Fig 4).

Within three weeks of waterlogging, leaf epinastic curvature was observed for waterlogged *E. leucophloia* and *E. terminalis* but not *E. victrix*. However, towards the end of the experiment this was observed for three *E. victrix* individuals. Between five and six weeks from waterlogging, 4 to 5 leaves of *E. terminalis* and *E. leucophloia* had turned a yellow-green colour (RHS Yellow Green Group C). Towards the end of the experiment, these leaves were bright yellow (RHS Yellow Group B). Many were dead and fell off the stem before harvesting. Most young leaves of waterlogged *E. victrix* seedlings were red-purple in colour (RHS Red Purple group A). In contrast, most of the newly flushed leaves from control seedlings were red (RHS Red Purple group A). Control plants had formed two to three small branches from the main stem, but no branches were produced on waterlogged seedlings for all three *Eucalyptus* species.

Stem hypertrophy (swelling) was observed only for waterlogged *E. victrix*. Three weeks after waterlogging, adventitious roots had also formed on the submerged portion of most *E. victrix* stems, and these floated just below the water surface. In contrast, neither *E. leucophloia* or *E. terminalis* waterlogged plants produced any adventitious roots. In most waterlogged plants, roots surrounded by soil had turned dark black. Most *E. victrix* waterlogged plant roots were decayed, with some remaining old roots being soft and presumed dead. During the harvest (65 days after flooding), *E. terminalis* and *E. leucophloia* water-logged plant roots were black.

Discussion

The genus *Eucalyptus* has adapted to different ranges of climatic and edaphic factors. Whereas no species grows in a permanently waterlogged condition, a few are able to grow in soil with some degree of waterlogging (Ladiges & Kelso 1977). The present study confirms the importance of adventitious root formation, at least in small *E. victrix*, as an adaptation to waterlogging. In addition, stem hypertrophy may also be considered an important adaptation in *E. victrix*. Production of adventitious roots was reflected in both rates of transpiration and stomatal conductance (Gomes & Kozlowski 1980a,b). Neither *E. terminalis* nor *E. leucophloia* produced any adventitious roots or showed stem hypertrophy when waterlogged. These species are presumed to be intolerant of waterlogging, as flood intolerant species seldom show any morphological changes and no recovery of gas exchange during the period of waterlogging (Tang & Kozlowski 1982). Species tolerant to the waterlogged condition often show reduced stomatal conductance and transpiration but recovered immediately after adventitious roots are formed (Gomes & Kozlowski 1980a,b). Hypertrophy (swelling) of the submerged portions of stems of *E. victrix* may assist with excretion of accumulated toxic compounds from the system (Gomes & Kozlowski 1986b). Production of adventitious roots may compensate for the death of older roots (Gomes & Kozlowski 1980a; Tsukahara & Kozlowski 1985).

Although no deaths occurred, waterlogging of *E. terminalis* and *E. leucophloia* reduced dry mass of both shoot and root. For both species, tips of lateral and tap roots had become very soft and some were visibly decaying. In addition, progressive decolouration was observed in a considerable number of leaves. It is emphasised that visual effects of waterlogging on these two species only started to become evident after 8-9 weeks. The rank of most flood tolerant to least tolerant is *E. victrix* > *E. leucopholia* > *E. terminalis*.

Adaptation to flooding by *E. victrix* is a combination of both morphological changes and responses (such as formation of adventitious roots and stem hypertrophy) and physiological adjustments (such as early stomatal closure and reduced transpiration rate). Further research is needed to study the effects of longer-term waterlogging on *E. terminalis* and *E. leucopholia*. Their absence from the floodplain is partially explained by the extent of detrimental effects and poor recovery following a period of waterlogging.

The process of adaptation to flooding by a single plant may require some time and transplanting shock is difficult to quantify (Megonigal & Day 1992). The use of seedlings of different ages clearly minimises initial root and shoot imbalances. The younger sets were of necessity not as well buffered to cope with flooding as seen for the lower root weights than shoot weights in 4- and 8-week seedlings compared with older sets. Established potted transplants of *E. victrix* appear to require 10-15 days for physiological adjustments. Younger, 14 cm height (13-week plants) are more sensitive than 19 cm height (17-week plants) in this respect. The relatively small mean height changes for all seedlings suggest that pot volume probably limited the expression of foliage material in the present study, whether plants were flooded or not.

Flooding may lead to much of the pre-existing root system being replaced with a new, morphologically distinct system (Hook 1984). Whether flood tolerant species can make continuous adjustments in root systems in response to periodic flooding is an interesting issue. Adventitious root development and intercellular air spaces are common responses to flooding. Adventitious roots are believed to confer tolerance to flooding (Gomes & Kozlowski 1980a; Kozlowski *et al.* 1991). Initiation of these roots is correlated with growth improvement in *E. camaldulensis* (Gomes & Kozlowski 1980a). Interestingly, the adventitious root material floated above the soil in the water tanks, although it is unclear whether this would be a useful adaptation in the field. Flooding of the Fortescue River valley is accompanied by considerable soil movement and it is possible that receding floodwaters could drop silt/clay material over any surface roots developed during a flood.

More detailed physiological analyses may have shown a change in transpiration rate in older flooded seedlings closer to the time at which adventitious roots had become visible, as seen for 4-month old *Fraxinus pennsylvanica* flooded seedlings (Gomes & Kozlowski 1980b). The production of adventitious roots is mainly due to flooding injury but may also be associated with ethylene production. Production of ethylene in unflooded plants has been associated with leaf epinasty (Denny & Miller 1935) and stem thickening (Zimmermann & Hitchcock 1933). Artificial application of ethylene releasing chemicals produces symptoms similar to those of flooding (Abeles 1973; Kawase 1974). Increased stem diameter may follow absorption of water by the bark. In *Eucalyptus globulus*, 10 days after flooding, the submerged portion of the stem had swollen (Gomes & Kozlowski 1980a). Similar observations apply to *E. viminalis*, *E. ovata* and *E. robusta* (Ladiges & Kelso 1977; Clemens & Pearson 1977).

Plant reactions to waterlogging vary with the duration, season, and tolerance to the stress (Ranney & Bir 1994). Root to shoot ratios may be expected to decrease in response to prolonged flooding (Megonigal & Day 1992). Although flooding time was equally long for all aged seedlings tested, *E. victrix* root/shoot ratios increased in the 17-week plants and decreased in response to flooding. In flooded conditions the soil becomes oxygen limited, in turn limiting physiological activity. Water and nutrient supplies through roots are reduced and the normal hormonal balance governing root shoot development can be disturbed (Kozlowski 1982). Flooded plants had a greater leaf area than control plants, at least for older plants, and leaf production did not appear inhibited by flooding. We hypothesised that a high seedling root/shoot ratio for *E. victrix* is an adaptation to drought. Despite its habitat being a floodplain, long dry periods occur each spring and longer droughts (with no flooding) are not uncommon. There is probably a change-over step when seedlings are large enough to permit sufficient bulk of new roots to replace those killed by the effects of flooding and this is associated with attained plant size at the onset of flooding. Alternatively, as all flooded plants had similar mean numbers of adventitious roots, perhaps the process simply requires more time in younger plants.

Observation suggests that seed dispersal of *E. victrix* occurs mainly in hot weather in summer (February to March), coincident with the greatest likelihood of rain (Florentine 1999). When capsules are ripe they dry rapidly, shedding the light seed that may blow some distance away. Germination is rapid at an optimum temperature of ~35-40 °C (Doran & Boland 1984) and is mainly effective in seedling production when seed falls onto moist sites just drying out from summer rain or flooding. The possibility of further flooding is high and this may lead to seedling submergence. The interest in this study was whether small seedlings of *E. victrix* can survive flooding. Field evidence suggests that established plants of 1-2 m can survive flooding but also that large numbers of summer germinating seedlings do not survive the following dry spring. The evidence obtained here suggests that seedlings of this species are remarkably tolerant of flooding, although total immersion has not been trialled.

In conclusion, there were significant difference in flood adaptations and response in gas exchange of 13- and 17- week old seedlings. In contrast, another two eucalypts were affected by flooding. This may explain why these species are absent from the floodplain area where *E. victrix* forms a unique patch of woodland. However, the relative impact of drought on *E. victrix* seedlings and the other species studied in this experiment has not been well documented. It will be worthwhile to examine those species and how they response to drought conditions.

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